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#### **REMARKS**

Claims 15-22 and 24-26 are pending in the application. Claims 1-14, 23 and 27-34 are withdrawn from consideration as being drawn to non-elected inventions. Applicants expressly reserve the right to file divisional applications or take such other appropriate measures deemed necessary to protect the inventions in the remaining claims.

Claims 15-22 and 24-26 are being examined to the extent they read on SEQ ID NO: 3. The claims have been amended to remove reference to non-elected sequences. Applicants expressly reserve the right to file divisional applications or take such other appropriate measures deemed necessary to protect the invention as it pertains to the remaining sequences.

Claims 15, 16, 19, 24, 25 and 26 (and thus dependent claims 17, 18, and 20-22) have been amended. Support for the amendments can be found in the specification as filed. No new matter has been added by way of amendment. Reexamination and reconsideration of the claims are respectfully requested.

### Sequence Identifiers

In the previous office action of November 27, 2002, the Examiner stated that Figures 4 and 5 were missing sequence identifiers. Applicants submitted proposed drawing changes with their response of December 16, 2002, and advised the Examiner that formal drawings in compliance with 37 CFR §1.84, including those changes, would be filed upon approval of the amendments by the Examiner and upon notification of allowable subject matter. In the instant Office Action, the Examiner requires the amendment of the Brief Description of the Drawings for figures 4 and 5. Applicants have made these required amendments and again assure the Examiner that formal drawings in compliance with 37 CFR §1.84 will be filed upon approval of the amendments by the Examiner and upon notification of allowable subject matter

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### Abstract

The Examiner has objected to the abstract of the invention as not being descriptive of the instant invention. Applicants have amended the abstract as shown in the previous section entitled "Amendments to the Specification", beginning on page 2 of the instant response. Applicants assert that the new abstract is clearly indicative of the invention to which the claims are directed. No new matter is added by way of the amendment to the abstract.

### Title

The Examiner has objected to the title of the invention as not being descriptive of the instant invention. Applicants have amended the title to "SCLEROTINIA-INDUCIBLE LIPID-TRANSFER POLYNUCLEOTIDES AND THEIR USES IN PLANT DISEASE RESISTANCE", in order to more clearly indicate the invention to which the claims are directed. This amendment is shown in the previous section entitled "Amendments to the Specification", beginning on page 2 of the instant response. No new matter is added by way of the amendment to the title.

### Hyperlinks

The Examiner has objected to the disclosure due to the inclusion of a hyperlink on page 13, lines 18-19. Applicants have amended the specification to remove the hyperlink, thereby obviating this objection.

### Claim Objections

The Examiner has objected to claims 15 and 25 because they contain non-elected sequences. Applicants have amended claims 15 and 25 to remove reference to non-elected sequences. The Examiner has also objected to claim 16 because there is an improper article before "nucleotide" in line 1. Applicants have amended claim 16 to correct the article. In view of these amendments, Applicants respectfully request that the claim objections be withdrawn.

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# The Rejection of Claims Under 35 U.S.C. §112, First Paragraph, Should be Withdrawn Enablement

The Examiner has rejected claims 15-22 and 24-26 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully disagree.

The Examiner states that the instant specification fails to provide guidance for lipid transfer protein-encoding nucleic acids with 70% identity to SEQ ID NO: 3, nucleic acids comprising at least 16 contiguous nucleotides of a nucleic acid that encodes SEQ ID NO: 4, or nucleic acids that hybridize to the latter nucleic acids. The Examiner further states that the instant specification fails to provide guidance for exact hybridization or amplification conditions and probes/primers to use in isolation of nucleic acids other than SEQ ID NO: 3. Applicants respectfully disagree.

Applicants have provided extensive guidance to be used in the selection of stringency conditions based on the desired outcome. See page 10, line 4 through page 11, line 26 of the specification as originally filed. Applicants have also provided an extensive discussion of specific exemplary low, medium and high stringency hybridization conditions and requirements on page 10, lines 14-27. The design of appropriate primers has been outlined by the Applicants on page 25, lines 3 to 14. Furthermore, Applicants have given guidance regarding the design and use of probes on page 25, line 15 through page 26, line 11. In view of the extensive guidance provided, Applicants assert that the invention is clearly enabled as claimed. However, in order to further prosecution, Applicants have amended claims 15 and 25 to remove reference to nucleic acids comprising 16 contiguous nucleotides of SEQ ID NO: 3. Applicants have also amended claims 15 and 25 to recite specific high stringency hybridization conditions as provided in the specification as filed, specifically as given on page 10, lines 24 to 27.

The Examiner states: "The specification ... suggests making variant nucleic acids by making conservative substitutions in the encoded protein. However making such 'conservative' substitutions ... does not produce predictable results." In support of this rejection, the Examiner cites Lazar *et al.* who teach that a conservative substitution reduced biological function while

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"nonconservative" substitutions had no effect. Likewise, the Examiner cites Hill *et al.* who teach that ADP-glucose pyrophosphorylase proteins mutated to substitute arginine for histidine (a "conservative" substitution), reduced the enzymes activity. The Examiner concludes that "all these mutated proteins however, would have at least 95% identity to the original protein and the nucleic acids encoding all these mutated proteins would hybridize under high stringency to the nucleic acids encoding the original protein."

Present claim 15 and 25 recite: "... a nucleotide sequence having at least 95% identity with SEQ ID NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity ...." (emphasis added). It is respectfully pointed out that non-operative embodiments are not claimed. Only those variants having lipid transfer activity are claimed.

The specification clearly states on page 23, lines 13-16: "Guidance as to appropriate amino acid substitutions that *do not affect desired biological activity* of the native protein may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Nat'l Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferable.." (emphasis added). The disclosure plainly acknowledges that conservative substitutions per se, *may* not produce a *functional* protein, but is one of many tools the skilled artisan may use to produce a nucleic acid of the currently claimed invention.

Applicants wish to point out that both references use the known homology to related proteins to identify and target particular amino acid residues. These references use homology to predict important conserved amino acids where substitution with another amino acid would actually be likely to have an impact on the activity of the protein. For example, Lazar *et al.* shows that even conservative substitution of L48 with similar amino acids (M or I) dramatically impacted activity, as predicted by the observed absolute conservation of leucine (L) at this position. In all cases, the modified protein had to be screened for the effect(s) of the modification. Clearly, one of skill in the art does believe that structural identity, as well as the presence of functional domains and conserved motifs are predictive of polypeptide function, as is clearly demonstrated by the pervasive use of sequence searching algorithms such as BLAST,

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FASTA, and the like, and multiple sequence alignment programs such as CLUSTAL and PileUp, and the like. Accordingly, one of skill in the art could use the known homology to related proteins to identify and target particular amino acid residues where substitution with another amino acid would actually be *unlikely* to have an impact on the activity of the protein.

The screening of a group of sequences containing from a few to many, inoperative species in order to isolate one or more operative species is a common practice in many aspects of the biotechnological arts. With the guidance provided in the specification as cited herein and in the previous responses, isolation of operative embodiments from a group of candidate sequences as claimed in present claims 15 or 25, clearly has a reasonable expectation of success by one skilled in the art.

The Examiner states that it is not clear that the instant nucleic acid actually encodes an LTP involved in a defense reaction. The Examiner states that a search of GenBank found no sequence in either the nucleotide or protein databases that matched the GenBank Accession Numbers listed on page 58, lines 4-12 of the specification. Applicants note that in fact, the numbers provided in the specification as filed are SwissProt accession numbers, not GenBank accession numbers. Applicants have amended the specification to indicate that these are in fact SwissProt database accession numbers. Applicants attach printouts of each of these sequences from the SwissProt database in order to show that the sequence numbers and organisms from which the sequences originate were correctly identified in the specification as originally filed. In addition, one sequence was identified by its SwissProt locus identifier (NLTP-VIGUN) rather than its SwissProt accession number, which is Q43681. Two typographical errors also occurred in the SwissProt accession numbers. Both O64431 and O81135 were prefaced with a Q rather than an O. Applicants have amended the specification to correct these errors.

Applicants attach a print-out of each of the cited sequences as Appendix A, as well as a GAP alignment for each sequence compared to SEQ ID NO: 4 showing the % identity and % similarity scores using the GAP algorithm (Appendix B). Applicants also attach a multiple sequence alignment (Appendix C) showing the highly conserved nature of these lipid transfer proteins, particularly in the second half of the sequences. Identical amino acids are shown in yellow

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highlighting and highly conserved amino acids are shown in green highlighting. Applicants assert that these alignments clearly indicate the identity of SEQ ID NO: 4 as a lipid transfer protein.

The Examiner has also rejected claims 15-22 and 24-26 because the specification does not disclose a repeatable process to obtain the nucleic acids (of Patent Deposit No. PTA-2182) and it is not apparent if the nucleic acids are really available to the public. Applicants direct the attention of the Examiner to page 20, lines 7-13 of the specification as originally filed, which clearly states that the deposit of the nucleic acids was made at the Patent Depository of the American Type Culture Collection, Manassas, Virginia, on June 30, 2000, and assigned Patent Deposit No. PTA-2182. Furthermore, the specification clearly states that this deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The undersigned attorney for Applicants hereby states that the deposits will be irrevocably and without restriction released to the public upon the issuance of a patent.

### Written Description

The Examiner has rejected claims 15-22 and 24-26 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Examiner states that the Applicants have not, in fact, described lipid transfer protein-encoding nucleic acids with 70% identity to SEQ ID NO: 3, nucleic acids comprising at least 16 contiguous nucleotides of a nucleic acid that encodes SEQ ID NO: 4, or nucleic acids that hybridize to nucleic acids comprising at least 16 contiguous nucleotides of a nucleic acid that encodes SEQ ID NO:4, and the specification fails to provide an adequate written description of the invention. Applicants respectfully disagree. In view of the claim amendments previously discussed, nucleic acids that comprise 16 contiguous nucleotides of SEQ ID NO:3 are no longer being claimed. Furthermore, nucleic acids with 70% identity to SEQ ID NO: 3 are also no longer being claimed.

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Applicants have given clear guidance of hybridization conditions and requirements as discussed *supra*. Furthermore, in order to expedite prosecution, Applicants have amended the claims to clearly specify the exact high stringency hybridization conditions required. As such, the written description is clearly adequate and commensurate with the claims.

Accordingly, Applicants request that the rejections of claims 15-22 and 24-26 under 35 U.S.C. §112, first paragraph, be withdrawn.

### The Rejection of Claims Under 35 U.S.C. §112, Second Paragraph, Should be Withdrawn

The Examiner has rejected claims 15-22 and 24-26 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention.

Applicants have amended claims 15 and 25 to remove part (d).

Applicants have also amended claims 15 and 25 to specifically recite highly stringent conditions, supported by the specification as originally filed on page 10, line 4 through page 11, line 26, more specifically on page 10, lines 24 to 27.

Applicants have amended claims 16, 19 and 24 to correct antecedent basis.

Applicants have amended claim 26 as suggested by the Examiner to clarify that the seed comprises the DNA construct of claim 25.

Accordingly, Applicants respectfully request withdrawal of the rejections of claims 15-22 and 24-26 under 35 U.S.C. §112, second paragraph.

### The Rejection of Claims Under 35 U.S.C. §102 Should be Withdrawn

The Examiner has rejected claims 15-21 and 24-26 under 35 U.S.C. 102(b) as being anticipated by Kragh *et al.* (WO 95/11306). Kragh *et al.* teach isolated nucleic acids comprising 21 contiguous nucleotides of a nucleic acid that encodes SEQ ID NO:4. In view of the claim amendments removing reference to isolated nucleic acids comprising at least 16 contiguous nucleotide segments of SEQ ID NO: 3, Applicants assert that the Kragh *et al.* reference no longer applies as 35 U.S.C. 102(b) art.

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The Examiner has rejected claim 15 under 35 U.S.C. 102(a) as being anticipated by Or *et al.* (2000, GenBank Accession No. AF195867), based on the Examiner's suggestion that the nucleic acid sequence taught by Or *et al.* would hybridize to SEQ ID NO: 3 under "stringent conditions." Applicants respectfully disagree. Applicants present a GAP alignment of AF195867 compared to SEQ ID NO: 3, showing that the percent identity between these two sequences is 46%. Applicants assert that under the highly stringent conditions recited in amended claim 15, AF195867 would not hybridize to SEQ ID NO: 3 due to its relatively low homology. Accordingly, Applicants assert that claim 15 is not anticipated by the nucleic acid sequence taught by Or *et al.* 

The Examiner has rejected claims 15-20, 22 and 24-26 under 35 U.S.C. 102 (b) as being anticipated by Dixon *et al.* (WO 98/51801) because Dixon *et al.* teach an isolated nucleic acid that would hybridize under "stringent conditions" to SEQ ID NO: 3 or nucleic acids that comprise at least 16 contiguous nucleotides of a nucleic acid encoding SEQ ID NO: 4.

Applicants present a GAP alignment of SEQ ID NO: 1 from Dixon *et al.* compared to SEQ ID NO: 3, showing that the percent identity between these two sequences is 40.6%. Applicants also note that there are no identical regions of 16 contiguous nucleotides or more between the two sequences. Applicants assert that under the highly stringent conditions recited in amended claims 15 and 25, SEQ ID NO: 1 from Dixon *et al.* would not hybridize to SEQ ID NO: 3 due to its relatively low homology. Accordingly, Applicants assert that claims 15-20, 22 and 24-26 are not anticipated by the nucleic acid sequence taught by Dixon *et al.* 

In view of the preceding remarks and claim amendments, Applicants respectfully request withdrawal of the rejections of the claims under 35 U.S.C. §102.

### CONCLUSION

In view of the above amendments and remarks, Applicants submit that the rejections of the claims under 35 U.S.C. §§112, first and second paragraphs and 35 U.S.C § 102(a) and (b) have been overcome. Applicants respectfully submit that this application is now in condition for allowance. Early notice to this effect is solicited.

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If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject Application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR §1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-1852.

Respectfully submitted,

Louise A. Foutch

Registration No. 37,133

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Johnston, Iowa 50131-1000

Phone: (515) 248-4835 Facsimile: (515) 334-6883

### Appendix A

```
92 AA.
    081135
                 PRELIMINARY;
                                 FRT;
ID
    081135;
AC
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ĐΤ
     01-NOV-1998 (TrEMBLrel. 08, Last sequence update)
ΙT
    01-OCT-2002 (TrEMBLrel. 22, Last annotation update)
E:T
    7 kDa lipid transfer protein.
ĽΞ
GN
    LTP-NE.
0:S
     Hordeum vulgare (Barley).
    Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
( )( )
    Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae;
QC.
OC
    Triticeae: Hordeum.
\bigcirc\times
    NCBI TaxID=4513;
F.11
     [1]
P.P
     SEQUENCE FROM N.A.
F.C
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    Chen F., Foolad M.R.;
P.A
     "Nucellar cell-specific expression of a lipid transfer protein gene in
RΤ
F.T
     barley.";
     Submitted (DEC-1997) to the EMBL/GenBank/DDBJ databases.
F.L
\mathbb{DR}
    EMBL; AF039024; AAC28263.1; -.
    InterPro; IPH003612; AAI.
DR
DR
    Pfam; PF00234; tryp_alpha_amyl; 1.
DR
    SMART; SM00439; AAI; 1.
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     COYAKDPALG RYITSPHARD TLLSCGLAVP RC
//
```

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                                   PRT;
                                             96 AA.
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A. `
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DT
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ΞT
     01-NOV-1996 (TrEMBLrel. 01, Last sequence update)
     01-OCT-2000 (TrEMBLrel. 22, Last annotation update)
ΞΞ
    Lipid transfer protein.
311
     LTP-2.
03
    Oryga sativa (Rice).
(j) ~
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\bigcirc\bigcirc
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Qu]
     Ehrhantoideae; Oryneae; Oryza.
ON
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PN
    [1]
ЭP
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ΞC
     STRAIN=IR36;
\Xi X
     MEDLINE=98316705; PubMed=9654133;
    Garcia-Garrido J.M., Menossi M., Puigdomenech P.,
21/1
    Martinez-Izquierdo J.A., Delseny M.;
F.A
ET
    "Characterization of a gene encoding an abscisic acid-inducible type-2
F.T
    lipid transfer protein from rice.";
RL
    FEBS Lett. 428:193-199(1998).
    EMBL; U16721; AAC50030.1; -.
\mathbb{D}(\mathbb{P})
DP.
    Gramene; Q40631; -.
DF.
    InterPro; IPR003612; AAI.
DR
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DR
     SMART; SMOC439; AAI; 1.
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11
```

```
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     01-AUG-1998 (TrEMBLrel. 07, Last sequence update)
[\cdot]
     01-OCT-2002 (TrEMBLrel. 22, Last annotation update)
\Gamma \cdot \Gamma
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ĿΕ
     PEC=1.
\Box\Box
O(3)
     Brassica dampestris (Field mustard).
     Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
(0)
     Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; Rosidae;
OC
     eurosids II; Brassicales; Brassicaceae; Brassica.
CH?
(:::
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F.:1
     [1]
F^{\prime}F^{\prime}
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E/C
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     MEDLINE=98138560; PubMed=3539157;
F.:
FA
     Toriyama K., Hanaoka K., Okada T., Watanabe M.;
ET
     "Molecular cloning of a cDNA encoding a pollen extracellular protein
F:T
     as a potential source of a pollen allergen in Brassica rapa.";
     FEBS Lett. 424:234-238(1998).
F:L
DP.
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D/FC
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DR
     SMART; SM00499; AAI; 1.
FΤ
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                    1
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SŲ
     SEQUENCE
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     PAFAQYISSP NSRKVLTACG IPYPSC
11
```

```
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     01-MOV-1996 (TrEMBLrel. 01, Last sequence update)
\Gamma^{\dagger}
     01-0CT-2002 (TrEMBLrel. 22, Last annotation update)
\Gamma
DΞ
     Putative nonspecific lipid transfer protein.
OS.
     Zinnia elegans.
     Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
()t]
\mathbb{C}^{\mathfrak{q}^{n}}
     Spermatophyta; Magnoliophyta; eudicctyledons; core eudicots;
ÇH,`
     Asteridae; campanulids; Asterales; Asteraceae; Asteroideae;
(\tilde{j})(\tilde{j})
     Heliantheae; Zinnia.
\bigcirc \mathbb{M}
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EII
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PΡ
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FIC
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F...
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     Ye I.H., Varner J.E.;
FΤ
     "Gene expression patterns associated with in vitro tracheary element
FΤ
     formation in isolated single mesophyll cells of Zinnia elegans.";
     Plant Physiol. 103:805-813(1993).
F:L
FIL
     SEQUENCE FROM N.A.
RΡ
RC
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RΑ
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FL
     Submitted (DEC-1994) to the EMBL/GenBank/DDBJ databases.
EII
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RC
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FΑ
     Demura T., Fukuda H.;
F^{\ast \square}
     "Molecular cloning and characterization of cDNAs associated with
F \cdot T
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FL
     Plant Physiol. 103:815-821(1993).
DΚ
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     EMBL; D30802; BAA06462.1; -.
DK
DΚ
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DR.
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DE
     SMART; SM00499; AAI; 1.
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//
```

```
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                                     PRT;
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     01-NOV-1996 (TrEMBLrel. 01, Last sequence update)
     01-OCT-2002 (TrEMBLrel. 22, Last annotation update)
\Gamma \cdot T
DΞ
     Lipid transfer protein (Fragment).
ŌΞ
     Senecio odorus.
00
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(n)
     Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots;
OC.
     Asteridae; campanulids; Asterales; Asteraceae; Asteroideae;
\mathbb{O}\mathbb{C}
     Senecioneae; Senecio.
([;]x[
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ET
     "Epidermis-specific transcripts including one that encodes a new class
P_{i}T_{j}
     of lipid transfer proteins in Kleinia odora.";
FL
     Submitted (JUN-1994) to the EMBL/GenBank/DDBJ databases.
DF:
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     InterPro; IPF.003612; AAI.
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DF.
     SMART; SM00499; AAI; 1.
PR
FΤ
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11
```

```
NLTP VIGUN
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AC
[:]
     15-JUL-1998 (Rel. 76, Created)
ΙT
     15-JUL-1998 (Rel. 36, Last sequence update)
     16-0CT-2001 (Fol. 40, Last annotation update)
[:T
     Probable nonspecific lipid-transfer protein AKCS9 precursor (LTP).
ĿΕ
0.5
    Vigna unquiculata (Cowpea).
\mathbb{Q}\mathbb{Q}
     Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
     Spermatophyta; Magnaliophyta; eudicotyledons; core eudicots; Rosidae;
\mathbb{C} \mathbb{C}
00
     eurosids I; Fabales; Fabaceae; Papilionoideae; Phaseoleae; Vigna.
\mathbb{C} \mathbb{N}
     NCBI TaxID=391/;
F.11
     [1]
ΡF
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FC
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     MEDLINE=94281682; PubMed=8012080;
F\Sigma
     Krause A., Sigrist C.J.A., Dehning I., Sommer H., Broughton W.J.;
FΑ
F:T
     "Accumulation of transcripts encoding a lipid transfer-like protein
FT'
     during deformation of nodulation-competent Vigna unquiculata root
F:T
     hairs.";
FL
     Mol. Plant Microbe Interact. 7:411-418(1994).
\hat{\mathbb{C}} \hat{\mathbb{C}}
     -!- FUNCTION: POTENTIAL LIPID TRANSFER PROTEIN.
CC
     -!- TISSUE SPECIFICITY: MOST TISSUES EXCEPT NODULES.
\mathbb{C}\mathbb{C}
     -!- DEVELOPMENTAL STAGE: EXPRESSION COFRELATES WITH ROOT HAIR
\mathbb{C}\mathbb{C}
         DEFORMATION.
CC
     -!- SIMILARITY: BELONGS TO THE FLANT LTP FAMILY.
CC
     ______
C\bar{C}
     This SWISS-PROT entry is copyright. It is produced through a collaboration
\mathbb{C}\mathbb{C}
     between the Swiss Institute of Bioinformatics and the EMBL outstation -
CC
     the European Bioinformatics Institute. There are no restrictions on its
CC
     use by non-profit institutions as long as its content is in no way
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     modified and this statement is not removed. Usage by and for commercial
CC.
     entities requires a license agreement (See http://www.isb-sib.ch/announce/
CC
     or send an email to license@isb-sib.ch).
CC
I:Ft
     EMPL; K79604; CAA56113.1; -.
ΞR
     InterPro: IPR003611; AAI.
DF:
     InterPro; IPR00052%; Plant LTP.
DE:
    lfam; PF00234; tryp alpha amyl; 1.
DR
    SMART; SM00499; AAI; 1.
DR.
    PROSITE; PS00597; PLANT LTP; FALSE NEG.
\mathbb{R}\mathbb{W}
     Lipid-binding: Transport; Signal.
FT
     WIGNAL 1 33 BY SIMILAPITY.
FT
     CHAIN
                  34
                         44
                                   PROBABLE NONSPECIFIC LIPID-TRANSFER
FΤ
                                   PROTEIN ARCS9.
    BY SIMILAPITY.
BY SIMILAPITY.
FΤ
EΤ
F^{\prime\prime}
                                  BY SIMILAPITY.
                        414
ΕT
    DISULFID
                                   BY SIMILARITY.
                65
               99 AA; 10449 MW; B52615DFAC30AC30 CRC64;
     SEQUENCE
     MTMKMEMEMS VVCAVVVVAL FLIDVGPVAE AVTCNPTELS SCVPAITGGS KPSSTCCSKL
     KVQEPCLONY IKNPSLKQYV NSPJAKKVLS NCGVTYPNO
11
```

```
ID
    042158
                  PRELIMINARY;
                                     PRT;
                                             94 AA.
A
     Q42158;
D'T
     01-NOV-1996 (TrEMBLrel. 01, Created)
     01-NOV-1996 (TrEMBLrel. 01, Last sequence update)
DT
     01-0CT-2002 (TrEMBLrel. 22, Last annotation update)
DT
DΕ
     Lipid transfer protein.
03
     Arabidopsis thaliana (Mouse-ear cress).
     Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
()()
QC.
     Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; Rosidae;
\mathbb{C}\mathbb{C}
     eurosias II; Brassicales; Brassicaceae; Arabidopsis.
\mathbb{G}\mathbb{X}
     NCBI_TaxID=3703;
F.11
     [1]
F_{i}\,F^{i}
     SEQUENCE FROM N.A.
F\subset
     STRAIN=cv. Columbia; TISSUE=Dry seed;
     Raynal M., Grellet F., Laudie M., Meyer Y., Cooke R., Delseny M.;
FA
P.L.
     Submitted (OCT-1993) to the EMBL/GenBank/DDBJ databases.
EII
     [2]
P.P
     SEQUENCE FROM N.A.
FC
     STRAIN=ov. Columbia; TISSUE=Dry seed;
FA
    CNES:
     Submitted (NOV-1993) to the EMBL/GenBank/DDBJ databases.
F.L
     EMBL; Z27019; CAA81566.1; -.
DR
     EMBL; Z29852; CAA52820.1; -.
DE.
DR InterPro; IPRO03612; AAI.
DP.
    Pfam; EF00234; tryp alpha amyl; 1.
DR
     SMART; SM00499; AAI; 1.
                94 AA; 10009 MW; 60C65EE36EC00F92 CRC64;
SQ
     SEQUENCE
     MVKVMWVSVL ALAAAILLLT VPVAEGVTCS PMQLASCLAA MTSSSPQSEA CCTKLREQQP
     CLOGYMENPT LEQYVSSPNA RKVSNSCKIP SPSC
11
```

## Appendix B

### Appendix B - Gap Results

### SEQ ID NO: 4 vs. SwissProt 064431 Brassica rapa

GAP of: O64431\_Brassica\_rapa check: 4839 from: 1 to: 86 to: SEQ ID NO 4 check: 8038 from: 1 to: 97 Symbol comparison table: blosum62.cmp CompCheck: 1102 BLOSUM62 amino acid substitution matrix. Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919. Gap Weight: 8 Average Match: 2.778 Length Weight: 2 Average Mismatch: -2.248 Length: 100 Quality: 177 Ratio: 2.058 Gaps: Percent Similarity: 46.988 Percent Identity: 36.145 Match display thresholds for the alignment(s): = IDENTITY : = 2 . = 1 064431 Brassica rapa x SEQ ID NO 4 May 28, 2003 16:52 ... 1 ......ILLTLFPAPNEAADTNVEAACDPKQLQPCLAAITGG 36 1 MKAPTMICFLVAVIAAMMVFMGQLPAA...TAVTCNYMELVPCAGAISSS 47 37 GQPSGDCCAKLKEQQPCLCGFSKNPAFAQYISSPNSRKVLTACGIPYPSC 86 48 QPPSGSCCSKVREQRPCFCGYLRNPSLRQFVSPAAAQKIASQCGVSIPQC 97

### SEQ ID NO: 4 vs. SwissProt 081135 Hordeum vulgare

GAP of: 081135 Hordeum vulgare check: 642 from: 1 to: 92 to: SEQ ID NO 4 check: 8038 from: 1 to: 97 Symbol comparison table: blosum62.cmp CompCheck: 1102 BLOSUM62 amino acid substitution matrix. Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919. Gap Weight: 8 Average Match: 2.778 2 Average Mismatch: -2.248 Length Weight: Quality: 154 Length: Ratio: 1.674 Gaps: 1 Percent Similarity: 40.659 Percent Identity: 34.066 Match display thresholds for the alignment(s): = IDENTITY : = 2 O81135 Hordeum vulgare x SEQ ID NO 4 May 28, 2003 16:53 ... 1 MAMRKTKELLLVAMMLALVV.....AARAAPCEVGQLTVCMPAITTGAK 44 1 .MKAPTMICFLVAVIAAMMVFMGQLPAATAVTCNYMELVPCAGAISSSQP 49 45 PSGACCANLGAQQGCFCQYAKDPALGRYITSPHARDTLLSCGLAVPRC 92 50 PSGSCCSKVREQRPCFCGYLRNPSLRQFVSPAAAQKIASQCGVSIPQC 97

### SEQ ID NO: 4 vs. SwissProt Q40631 Oryza sativa

GAP of: Q40631\_Oryza\_sativa check: 1485 from: 1 to: 96 to: SEQ ID NO 4 check: 8038 from: 1 to: 97 Symbol comparison table: blosum62.cmp CompCheck: 1102 BLOSUM62 amino acid substitution matrix. Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919. Gap Weight: 8 Average Match: 2.778 Length Weight: 2 Average Mismatch: -2.248 Quality: 166 Length: 99 Ratio: 1.729 Gaps: Percent Similarity: 42.553 Percent Identity: 32.979 Match display thresholds for the alignment(s): = IDENTITY : = 2 . = 1 Q40631 Oryza sativa x SEQ ID NO 4 May 28, 2003 16:53 ... 1 MMRKLAVLVLAVAMVAAC...GGGVVGVAGASCNAGQLTVCAAAIAGGAR 47 1 .MKAPTMICFLVAVIAAMMVFMGQLPAATAVTCNYMELVPCAGAISSSQP 49 48 PTAACCSSLRAQQGCFCQFAKDPRYGRYVNNPNARKTVSSCGIALPTCH 96 50 PSGSCCSKVREQRPCFCGYLRNPSLRQFVSPAAAQKIASQCGVSIPQC. 97

### SEQ ID NO: 4 vs. SwissProt Q41378 Senecio odorus

GAP of: Q41378 Senecio odorus check: 6134 from: 1 to: 89 to: SEQ ID NO 4 check: 8038 from: 1 to: 97 Symbol comparison table: blosum62.cmp CompCheck: 1102 BLOSUM62 amino acid substitution matrix. Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad. Sci. USA 89: 10915-10919. Gap Weight: 8 Average Match: 2.778 Length Weight: 2 Average Mismatch: -2.248 Quality: 240 Length: Ratio: 2.697 Gaps: 0 Percent Similarity: 53.933 Percent Identity: 42.697 Match display thresholds for the alignment(s): = IDENTITY : = 2 Q41378 Senecio odorus x SEQ [D NO 4 May 28, 2003 16:55 ... 1 .....SAIYIVALLVMIVAGSKVATAATCSVTELMPCSSAFTSSAAP 42 1 MKAPTMICFLVAVIAAMMVFMGQLPAATAVTCNYMELVPCAGAISSSQPP 50 43 TAQCCTKLKEQSPCLCGYLKNPTLKQYITNPNAKKVTSTCGVPIPNC 89 51 SGSCCSKVREQRPCFCGYLRNPSLRQFVSPAAAQKIASQCGVSIPQC 97

### SEQ ID NO: 4 vs. SwissProt Q42158 Arabidopsis thaliana

GAP of: Q42158_Arabidepsis_thaliana check: 688 from: 1 to: 94 to: SEQ_ID_NO_4 check: 8038 from: 1 to: 97
Symbol comparison table: blosum62.cmp CompCheck: 1102 BLOSUM62 amino acid substitution matrix. Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. Proc. Natl. Acad.  Sci. USA 89: 10915-10919.
Gap Weight: 8 Average Match: 2.778 Length Weight: 2 Average Mismatch: -2.248
Quality: 230 Length: 99 Ratio: 2.447 Gaps: 2 Percent Similarity: 56.522 Percent Identity: 45.652
<pre>Match display thresholds for the alignment(s):</pre>
Q42158_Arabidopsis_thaliana x SEQ_ID_NO_4 May 28, 2003 16:55
46 PQSEACCTKLREQQPCLCGYMRNPTLRQYVSSPNARKVSNSCKIPSPSC 94     .  .  .       .  .

### SEQ ID NO: 4 vs. SwissProt Q43681 Vigna unguiculata

```
GAP of: Q43681 Vigna unguiculata check: 7095 from: 1 to: 99
to: SEQ ID NO 4 check: 8038 from: 1 to: 97
Symbol comparison table: blosum62.cmp CompCheck: 1102
BLOSUM62 amino acid substitution matrix.
Reference: Henikoff, S. and Henikoff, J. G. (1992). Amino acid
         substitution matrices from protein blocks. Proc. Natl.
Acad.
         Sci. USA 89: 10915-10919.
                    8
       Gap Weight:
                          Average Match: 2.778
     Length Weight:
                    2 Average Mismatch: -2.248
          Quality: 237
                                 Length:
                                          101
           Ratio: 2.443
                                   Gaps:
Percent Similarity: 55.789 Percent Identity: 45.263
      Match display thresholds for the alignment(s):
                = IDENTITY
                 : = 2
                 . = 1
Q43681 Vigna unguiculata x SEQ ID NO 4 May 28, 2003 16:57 ...
      1 MTMKMKMKMSVVCAVVVVALFLIDVG..PVAEAVTCNPTELSSCVPAITG 48
          1 ....MKAPTMICFLVAVIAAMMVFMGQLPAATAVTCNYMELVPCAGAISS 46
              49 GSKPSSTCCSKLKVOEPCLCNYIKNPSLKOYVNSPGAKKVLSNCGVTYPN 98
          47 SQPPSGSCCSKVREQRPCFCGYLRNPSLRQFVSPAAAQKIASQCGVSIPQ 96
     99 C 99
     97 C 97
```

## Appendix C

### Appendix C: Multiple Sequence Alignment Results

Symbol comparison table: blosum62.cmp CompCheck: 1102

GapWeight: 8
GapLengthWeight: 2

O64431\_Brass\_pileup\_185666.txt MSF: 100 Type: P May 20, 2003 16:48 Check: 4538 ...

	1				50
081135_Horde	~~~MAMRKTK	ELLL. AMM	ALVAAR	AAPCEVGQLT	VCMPAI GA
Q40631_Oryza	~~~~MMRK <b>A</b>	VLVLA AM A	ACGGG VGV	GA CNAGQLT	V <b>CAAA</b> I GGA
Q42158_Arabi					
Q41378_Senec					
Q42392Zin					
Q43681_Vigna	MTMKMKMK#S	V CAV VVA	FLEDVGPVAE	AVTCNPTELS	SCVPAI GGS
SEQ_ID_NO_4					
064431_Brass	~~~~~~~	~~~I LFP	APNEAAD NV	EA CDPKQLQ	PCLAAI GGG
	51				100
081135_Horde		GAQQGCFCQY	A DP LGR	TSPHA~RDTLL	SCG PRC~
Q40631_Oryza	RP A CCSS	RAQQGCFCQ	A DPRYGR V	NNPNA~RKTVS	SCGPTCH
Q42158_Arabi			RNPLRQV	SSPNA~RK	
Q41378_Senec					TCGVPIP C~
Q42392Zin				SSPNA~KK AN	ACGVPI PKC~
Q43681_Vigna		VQEPCLCNY		NSPGA~KK LS	
SEQ_ID_NO_4			LRNPSLRQFV		QCGVSIPQC~
064431_Brass	QPSGDCC K	EQQPCLCG	SINPIFAQ	SSPN ~RK	ACG PPSC~

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### THAT WHICH IS CLAIMED:

- 1. An isolated nucleic acid molecule having a nucleotide sequence for a promoter that is capable of initiating transcription in a plant cell, wherein said nucleotide sequence for said promoter is selected from the group consisting of:
- a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
- b) a nucleotide sequence selected from the group consisting of the sequences deposited as Patent Deposit No. PTA-2182;
- c) a nucleotide sequence comprising at least 30 contiguous nucleotides of the sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
- d) a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
- e) a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
- f) a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6; and
- g) a nucleotide sequence that hybridizes under stringent conditions to the complement of a sequence of a), b), or c).
- 2. A DNA construct comprising a nucleotide sequence of claim 1 operably linked to a heterologous nucleotide sequence of interest.
  - 3. A vector comprising the DNA construct of claim 2.
- 4. A host cell having stably incorporated in its genome the DNA construct of claim 2.
- 5. A method for inducing expression of a heterologous nucleotide sequence in a plant, said method comprising the steps of transforming a plant cell with a DNA construct comprising said heterologous nucleotide sequence operably linked to a

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promoter that is capable of initiating transcription in a plant cell in response to a stimulus, regenerating a stably transformed plant from said plant cell, and exposing said plant to said stimulus, wherein said promoter comprises a nucleotide sequence of claim 1.

- 5 6. The method of claim 5, wherein said plant is a monocot.
  - 7. The method of claim 5, wherein said plant is a dicot.
  - 8. The method of claim 7, wherein said dicot is sunflower.
  - 9. A plant cell stably transformed with a DNA construct comprising a heterologous nucleotide sequence operably linked to a promoter that is capable of initiating transcription in said plant cell, wherein said promoter comprises a nucleotide sequence of claim 1.
  - 10. A plant stably transformed with a DNA construct comprising a heterologous nucleotide sequence operably linked to a promoter that is capable of initiating transcription in a plant cell, wherein said promoter comprises a nucleotide sequence selected from the group consisting of:
  - a) a nucleotide sequence comprising the sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
  - b) a nucleotide sequence selected from the group consisting of the sequences deposited as Patent Deposit No. PTA-2182;
  - c) a nucleotide sequence comprising at least 30 contiguous nucleotides of the sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
    - d) a nucleotide sequence having at least 70% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
    - e) a nucleotide sequence having at least 80% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6;
- 30 f) a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:6; and

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- g) a nucleotide sequence that hybridizes under stringent conditions to the complement of a sequence of a), b), or c).
  - 11. The plant of claim 10, wherein said plant is a monocot.
  - 12. The plant of claim 11, wherein said plant is a dicot.
  - 13. The plant of claim 12, wherein dicot is sunflower.
- 10 14. Transformed seed of the plant of claim 10.
  - 15. An isolated nucleic acid molecule having a nucleotide sequence selected from the group consisting of:
    - a) the sequence set forth in SEQ ID NO:1 or SEQ ID NO:3;
  - b) a nucleotide sequence selected from the group consisting of the sequences deposited as Patent Deposit No. PTA-2182;
    - a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4;
  - d) a nucleotide sequence encoding the amino acid sequence encoded by a nucleotide sequence deposited as Patent Deposit No. PTA-2182;
    - e) a nucleotide sequence comprising at least 16 contiguous nucleotides of a nucleotide sequence of a), b), c), or d);
    - f) a nucleotide sequence having at least 70% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;
- g) a nucleotide sequence having at least 80% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;
  - h) a nucleotide sequence having at least 90% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;

a nucleotide sequence having at least 70% identity with SEQ ID

NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;

i)

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- j) a nucleotide sequence having at least 80% identity with SEQ ID NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;
- k) a nucleotide sequence having at least 90% identity with SEQ ID
   NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;
  - l) a nucleotide sequence that hybridizes under stringent conditions to the complement of a sequence of a), b), c), d), or e); and
- m) the complement of a nucleotide sequence of a), b), c), d), e), f), g), 10 h), i), j), k), or l).
  - 16. A DNA construct comprising a nucleotide sequence of claim 15 operably linked to a promoter that drives expression in a plant cell.
    - 17. A vector comprising the DNA construct of claim 16.
  - 18. A host cell having stably incorporated in its genome the DNA construct of claim 16.
- 20 19. A method for creating or enhancing disease resistance in a plant, said method comprising transforming said plant with a DNA construct comprising a nucleotide sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell and regenerating stably transformed plants, wherein said nucleotide sequence is selected from the nucleotide sequences of claim 15.
  - 20. The method of claim 19, wherein said plant is a dicot.
  - 21. The method of claim 20, wherein said dicot is sunflower.
- The method of claim 19, wherein said promoter is an inducible promoter.

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23. The method of claim 22 wherein said inducible promoter is selected from the group consisting of promoters for sunflower chitinase and sunflower LTP.

- om Jelekel
- 24. A plant cell stably transformed with a DNA construct comprising a nucleotide sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell, wherein said nucleotide sequence is selected from the nucleotide sequences of claim 15.
- 25. A plant stably transformed with a DNA construct comprising a nucleotide sequence operably linked to a promoter that drives expression of a coding sequence in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:
  - a) the sequence set forth in SEQ ID NO:1 or SEQ ID NO:3;
  - b) a nucleotide sequence selected from the group consisting of the sequences deposited as Patent Deposit No. PTA-2182;
  - c) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4;
  - d) a nucleotide sequence encoding the amino acid sequence encoded by a nucleotide sequence deposited as Patent Deposit No. PTA-2182;
  - e) a nucleotide sequence comprising at least 16 contiguous nucleotides of a nucleotide sequence of a), b), c), or d);
  - f) a nucleotide sequence having at least 70% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;
  - g) a nucleotide sequence having at least 80% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;
  - h) a nucleotide sequence having at least 90% identity with SEQ ID NO:1, wherein said nucleotide sequence encodes a polypeptide having chitinase activity;
  - i) a nucleotide sequence having at least 70% identity with SEQ ID NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;

- j) a nucleotide sequence having at least 80% identity with SEQ ID NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;
- k) a nucleotide sequence having at least 90% identity with SEQ ID
   NO:3, wherein said nucleotide sequence encodes a polypeptide having lipid transfer activity;
  - a nucleotide sequence that hybridizes under stringent conditions to the complement of a sequence of a), b), c), d), or e); and
- m) the complement of a nucleotide sequence of a), b), c), d), e), f), g), 10 h), i), j), k), or l).
  - Transformed seed of the plant of claim 25.
- 27. A substantially purified protein having an amino acid sequence selected from the group consisting of:
  - a) the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4;
  - b) an amino acid sequence encoded by the nucleotide sequence deposited as Patent Deposit No. PTA-2182;
- 20 c) an amino acid sequence that shares at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO:2, wherein said amino acid sequence has chitinase activity;
  - d) an amino acid sequence that shares at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO:4, wherein said amino acid sequence has lipid transfer activity;
  - e) an amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3; and
  - f) an amino acid sequence encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID
- 30 NO:1, wherein said amino acid sequence has chitinase activity; and

- g) an amino acid sequence encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence set forth in SEQ ID NO:3, wherein said amino acid sequence has lipid transfer activity.
- 5 28. A composition comprising the protein of claim 27 and a carrier.
  - 29. The composition of claim 28, wherein said carrier is selected from a surface active agent, an inert carrier, an encapsulating agent, and an agrochemical.
- The composition of claim 28, wherein said carrier is a pharmaceutical carrier.
  - 31. A method for controlling a plant pathogen, said method comprising applying an anti-pathogenic amount of the protein of claim 27 to the environment of said pathogen.
  - 32. The method of claim 31 wherein said anti-pathogenic amount of said protein is applied to a plant.
- 20 33. The method of claim 31 wherein said anti-pathogenic amount of said protein is applied by a procedure selected from the group consisting of spraying, dusting, scattering, and seed coating.
- 34. A method for controlling a plant pathogen comprising applying an antipathogenic amount of the composition of claim 28 to the environment of said pathogen.